Microfiltration and Ultraviolet Irradiation to Eliminate *Ceratomyxa shasta* (Myxozoa: Myxosporea), a Salmonid Pathogen, from Fraser River Water, British Columbia

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MICROFILTRATION AND ULTRAVIOLET IRRADIATION TO ELIMINATE *Ceratomyxa shasta* (Myxozoa: Myxosporea), A SALMONID PATHOGEN, FROM FRASER RIVER WATER, BRITISH COLUMBIA

by

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ABSTRACT

Bower, Susan M. and L. Margolis. Microfiltration and ultraviolet irradiation to eliminate Ceratomyxa shasta (Myxozoa: Myxosporea), a salmonid pathogen, from Fraser River water, British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 1364: 11 p.

Enteric infections of Ceratomyxa shasta developed in juvenile chinook salmon, Oncorhynchus tshawytscha, (from the Chehalis River hatchery, Fraser River) exposed to water pumped from the North Arm of the Fraser River, British Columbia. After about 6 days of exposure to this water in late June 1983, fish began to die with heavy C. shasta infections during week 4 of incubation in C. shasta-free water. However, by late July mortalities occurred within 2 weeks of incubation after 7 days of exposure. Ceratomyxa shasta was not detected in juvenile chinook salmon held for up to 48 days in turbid Fraser River water after filtration (25 µm filters of #16 silica sand) followed by ultraviolet irradiation (approximately 90,000 microwatts sec/cm2).

RÉSUMÉ

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Des infections entériques causées par Ceratomyxa shasta se sont développées chez des saumons quinnats juvéniles (Oncorhynchus tshawytscha) de la piscifacture de la rivière Chehalis (fleuve Fraser) exposés à de l'eau pompée du bras nord du fleuve Fraser (Colombie-Britannique). Après environ 6 d d'exposition à la fin de juin 1983, les poissons ont commencé à mourir de graves infections au C. shasta au cours de la quatrième semaine de stabulation en milieu libre de C. shasta. Toutefois, à la fin juillet, la mort est survenue en moins de deux semaines de stabulation après 7 d d'exposition. Le pathogène n'a pas été décelé chez des quinnats juvéniles gardés jusqu'à 48 d dans de l'eau turbide du fleuve Fraser filtrée (filtres de 25 µm de sable de silice #16) et irradiée aux ultra-violets (environ 90,000 microwatts sec/cm2).

INTRODUCTION

The myxosporean parasite Ceratomyxa shasta Noble, 1950 is pathogenic to juvenile salmonids, infections often resulting in high mortalities among exposed fish (Johnson et al. 1979; Ratliff 1983; Ching and Munday 1984a,b). This parasite has been identified in mature and postspawned salmonids (chinook salmon, Oncorhynchus tshawytscha; coho salmon, Oncorhynchus kisutch; steelhead trout, Salmo gairdneri; and cutthroat trout, Salmo clarki) throughout the Fraser River drainage basin (McDonald 1983; Arai and Mudry 1983). Ching and Munday (1984a) demonstrated the presence of infective stages of this parasite at several locations along the Fraser River by holding caged chinook salmon in the river. Recently, Bower (1985) found naturally infected chinook salmon smolts at the mouth of the Fraser River. The impact of this parasite on salmonid populations in the Fraser River is not known. However, in the summer of 1982, the majority of juvenile chinook salmon held in tanks supplied with water pumped from the North Arm of the Fraser River at Iona Island (Fig. 1) died of C. shasta infections before a 6 week long experiment designed to examine the effect of sewage effluent on the fish was completed.

Bedell (1971), Sanders et al. (1972), and Johnson et al. (1979) recommended the use of microfiltration and ultraviolet (U.V.) irradiation techniques to reduce the number of C. shasta infective stages in water. Since the experiment to study the effect of sewage effluent on juvenile chinook salmon was to be repeated again in the summer of 1983, we proposed to examine the infectivity of C. shasta to these fish exposed to water pumped from the lower Fraser River and the efficiency of microfiltration and U.V. irradiation in eliminating or reducing the number of C. shasta infective stages in the turbid Fraser River.

MATERIALS AND METHODS

In conjunction with experiments conducted at the Iona Sewage Treatment Plant (Fig. 1) to study the effect of sewage effluent on juvenile chinook salmon, tanks were set up to examine the effect of treated (microfiltered and U.V. irradiated) and untreated water pumped from the North Arm of the Fraser River on the infectivity of C. shasta. Three flow-through fiberglass tanks were used for this purpose. Two large tanks (each approximately 1,100 L) were each stocked with about 500 juvenile chinook salmon (77 to 108 mm fork length and 4.2 to 12.9 gm wet weight) from a hatchery located on the Chehalis River, a tributary of the Fraser River. One of these tanks was supplied with Fraser River water that was filtered (25 µm filters consisting of #16 silica sand) and treated with U.V. light (12 lamp unit, each lamp with 14.3 watts at 2537 A with a flow rate of 454 liters/min and 80-85% transmission, to give approximately 90,000 microwatts sec/cm^2). Approximately 25 fish were removed from this tank weekly between June 17 and July 21, 1983, transported live to the Pacific Biological Station (PBS) in

Nanaimo, and examined within two days for C. shasta. The fish were examined microscopically (250 X bright field) for motile trophozoites, sporoblasts, and spores of C. shasta by scanning wet mount preparations of ascites fluid (if present) and scrapings of the mucosa from the lower intestine for five minutes.

The second large tank was supplied with untreated Fraser River water pumped directly from the river. Each week approximately 50 fish were removed from this tank and transported live to PBS in Nanaimo. Twenty five fish were examined for C. shasta within two days and 25 fish were place in 50-L flow-through fiberglass tanks supplied with approximately 2 L of fresh, C. shasta-free water per minute. The water temperature at the PBS holding facility was about 14°C in June and increased to 18°C by mid-August. The salmon were fed Oregon moist pellets (OMP) daily. The date of death and results of a postmortem examination for C. shasta were recorded for each fish.

The third smaller tank (approximately 50 L in volume) received untreated Fraser River water and was restocked about once a week between June 17 and August 4 with about 25 juvenile chinook salmon from the same stock used in the large tank. However, these fish had been maintained in C. shasta-free water at the West Vancouver Laboratory (Fisheries Research Branch, Department of Fisheries and Oceans) until required. At the end of each week the exposed chinook salmon were transported to PBS in Nanaimo and maintained in the same manner as described for the 25 fish from the second large tank until they died. The last two groups (26 and 23 fish) were exposed to Fraser River water for 14 and 11 days, respectively.

RESULTS

None of 154 juvenile chinook salmon held in Fraser River water treated by microfiltration and with U.V. light became infected with C. shasta (Table 1). However, after two weeks of exposure to untreated Fraser River water, one of 25 chinook salmon had a few presumably C. shasta trophozoites in its lower intestine. By week 4, 17 of 25 chinook salmon harboured presumed C. shasta trophozoites and by week 5, all 18 chinook salmon sampled were infected and half of them had C. shasta spores (Table 1).

All 263 chinook salmon exposed to untreated Fraser River water for one or more weeks and subsequently incubated in C. shasta-free water died (Table 2). Sixty-four (24%) of the fish died with advanced C. shasta infections. One hundred thirty seven (52%) died before the C. shasta infection was sufficiently advanced for the production of sporoblasts and spores. However, the intestinal mucosa of these fish was haemorrhagic and active ameboid trophozoites were identified in wet mount preparations of intestinal scrapings from recently dead fish. Sixty-two (24%) fish died without evidence of C. shasta in the microscopically examined intestinal scrapings and thus probably succumbed to other causes. Bacterial analysis of some moribund fish indicated the presence of systemic infections with

myxobacteria and Aeromonas salmonicida, the aetiological agents of cold water disease and furunculosis, respectively (G. E. Hoskins personal communication). Although the fish had been treated while at Iona Island or at the West Vancouver Laboratory with terramycin (3 gm/45 kg of fish/day for 10 days) incorporated into the diet, some still succumbed to these salmonid pathogens.

Mortalities due to C. shasta among juvenile chinook salmon exposed to untreated Fraser River water for 6 days in mid-June did not appear until the fourth week of incubation in uncontaminated water (Table 2). By mid-July, mortalities due to C. shasta infection occurred as early as the second week of incubation after 7 days of exposure. By early August, 11 days of exposure were sufficient to result in mortalities from C. shasta during the first week of incubation in C. shasta-free water.

Chinook salmon with 13 to 20 days of continuous exposure to untreated Fraser River water from mid-June to early July showed mortalities within the second week of incubation or within 4 and 5 weeks of the beginning of the experiment (Table 2). All 25 fish returned to Nanaimo for incubation in uncontaminated water after 27 days of exposure to Fraser River water beginning in mid-June died within the first week.

Temperatures of the Fraser River water pumped into the experimental tanks increased from 14 to 20°C between June 17 and Aug. 15. Throughout this period, the water was turbid, carrying about 2.2×10^6 particles per ml prior to filtration and about 1.3×10^6 particles per ml after filtration, with the majority of the particles (1.2×10^6/ml) in the filtered water being less than 5 µm in diameter.

DISCUSSION

Early stages of C. shasta in salmonid hosts have been described as granular, multinucleate, and non-ameboid. Slightly later stages appear as active, binucleate, ameboid trophozoites (Schafer 1968). In the present study, the earliest identifiable stage in wet mount preparations of fresh material was the active ameboid trophozoite. Without staining, the number of nuclei could not be determined and nonmotile forms could easily be confused with host cells such as lymphocytes and macrophages. In addition to the ameboid trophozoites, some infected fish harboured small round cells (approximately 10µm in diameter) from which many protoplasmic spikes radiated (similar in shape to a radiolarian without a skeleton). These cells were never observed in noninfected fish. However, without using techniques such as immunofluorescence for species identification, the identity of nonmotile cells could not be confirmed. As indicated by Johnson et al. (1979) and Ratliff (1981), the diagnosis of C. shasta requires that typical spores be found and identified by their size and shape. In the present study, some of the fish

examined did not have infections advanced to the spore stage. However, all material examined was fresh and the characteristic ameboid movements of the trophozoites were distinctive. Thus, fish in which only motile trophozoites were found were considered positive for C. shasta.

In addition to the two lower Fraser River localities (km 69 at Whonnock and km 102 at Harrison) identified by Ching and Munday (1984a) as locations where infective stages of C. shasta occur, a third location, close to the mouth of the North Arm of the Fraser River, adjacent to the Iona Sewage Treatment Plant was identified in the present study. Water pumped from this location from at least mid-June to late July contained sufficient infective forms of C. shasta to infect juvenile chinook salmon after only 7 days of exposure (Table 2).

Zinn et al. (1977) demonstrated that of six stocks of chinook salmon from the Columbia River system where C. shasta is known to be enzootic, five were highly resistant to the lethal effects of C. shasta, whereas of four stocks from Oregon coastal rivers, where C. shasta apparently does not occur, only one showed some resistance. The juvenile chinook salmon used in the present study were from the Chehalis River hatchery, on a tributary of the Fraser River. During downstream migration, this stock of chinook salmon would have to pass through the lower Fraser River where C. shasta is enzootic. Nevertheless, in our experiments, chinook salmon from this stock suffered high mortalities caused by C. shasta. The experimental exposures to infective stages of C. shasta may have been far greater than juvenile chinook smolts would normally encounter while migrating down the Fraser River to the ocean. However, as shown in the preliminary study by Bower (1985), not all Fraser River chinook smolts make it to the estuary without becoming infected. Bower observed C. shasta sporoblasts or spores in 2 of 28 feral juvenile chinook salmon captured in the Fraser River estuary.

Bedell (1971) and Sanders et al. (1972) found that U.V. treatment of water was effective for controlling infective stages of C. shasta. However, Sanders et al. (1972) recommended water filtration prior to U.V. irradiation because U.V. light has poor penetrating power and efficiency is greatly reduced by turbidity. In both experiments conducted by Bedell (1971) and one experiment conducted by Sanders et al. (1972) the U.V. treated water was clear. Although turbidity measurements were not given, the water used in the second experiment of Sanders et al. (1972) was reported to have a wide diversity of particles varying in size from about 1µm to macroscopic after sand filtration. However, dosages of 360,000 microwatt-sec/cm^2 were found effective for inactivating infective stages of C. shasta. In the present study, the filtration and U.V. dosage of 90,000 microwatt-sec/cm^2 eliminated the infective forms or greatly reduced their numbers. For microbial disinfection, Fox (1982) recommended a minimum dosage of 16,000 microwatt-sec/cm^2 at all points throughout the water column, but this dosage may be too low for inactivating C. shasta, especially if the water is turbid.

None of the chinook salmon held for 48 days in Fraser River water treated by microfiltration and U.V. irradiation became infected with C. shasta thus allowing the experiments on the effect of sewage effluent on juvenile chinook salmon to be conducted. However, two things could have been done to make the water treatment experiments more conclusive. First, a non-Fraser

River chinook salmon stock known to be very susceptible, such as that from the Capilano River hatchery in British Columbia (Chiny 1984; Chiny and Munday 1984b), should have been used in addition to the Chehalis River stock. The Chehalis River chinook salmon stock may have some degree of resistance inherited from ancestors that survived exposure to this parasite and therefore may have some ability to withstand low-level exposures as might happen after U.V. treatment of turbid water. Secondly, even in highly susceptible stocks, low-level exposures may require a much longer incubation time before infections are evident (Ratliff 1983). An incubation period of 48 days may have been insufficient to detect infections, but fish from treated water were not available beyond 48 days because the remaininy fish were required for the sewage effluent studies.

To date, there is no chemotherapeutic drug effective in preventiny or treatiny ceratomyxosis. The antibiotic terramycin, used by other workers (Udey et al. 1975; Zinn et al. 1977; Ratliff 1981) to control various bacterial pathogens, was incorporated into the diet of chinook salmon experimentally exposed to water pumped from the Fraser River at Iona. Nevertheless, some of the fish died without evidence of C. shasta infection and a few of these were shown to be systemically infected with myxobacteria and Aeromonas salmonicida. Thus, the cause of death of the experimental chinook salmon could not be attributed solely to C. shasta. However, survival time of C. shasta-infected fish seems to be reduced as the summer advances (Table 2). Udey et al. (1975) and Ratliff (1981) found that incubation times were reduced at elevated temperatures. In the present study, incubation and river temperatures were between 14 and 16°C in June and increased to 18 and 20°C in August, which may explain the shorter time to death with the progression of summer. However, more infective forms may also be present in the water supply as the summer advances thereby increasing the size of the infecting dose, and thus, perhaps, reducing the survival time. The only described method for quantifyiny the number of infective forms present in a water supply is indirect and involves placiny susceptible fish in groups of doubling numbers from 5 to 160 and incubatiny them in a standard volume of suspect water followed by quantifyiny the prevalence of infection in these fish after about 100 days incubation (Ratliff 1983). In the present study, this procedure was not tested.

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Table 1. Effect[a] of filtration and ultraviolet treatment[b] of Fraser River water on occurrence of *Ceratomyxa shasta* infection in juvenile *Oncorhynchus tshawytscha*. (Fish were examined within two days after the period of exposure.)

Date of exposure termination (1983)	Period of exposure (weeks)	Fish from treated water	Fish from untreated water
June 17	0	$\frac{0+0}{30}$	-
June 23	1	$\frac{0+0}{24}$	$\frac{0+0}{25}$
June 30	2	$\frac{0+0}{25}$	$\frac{1+0}{25}$
July 7	3	$\frac{0+0}{25}$	$\frac{0+0}{26}$
July 14	4	-	$\frac{17+0}{25}$
July 21	5	$\frac{0+0}{25}$	$\frac{9+9}{18}$
Aug. 4	7	$\frac{0+0}{25}$	-

[a]Recorded as: $\frac{\text{number of fish with trophozoites only} + \text{number of fish with trophozoites and sporoblasts, and/or spores}}{\text{total number of fish examined}}$

[b]Filtration through 25 µm filters consisting of #16 silica sand and U.V. exposure through units consisting of 12 lamps (14.3 watts at 2537 Å per lamp) at a flow rate of 454 L/min with 80 to 85% transmission to give approximately 90,000 microwatt-sec/cm^2.

Table 2. Mortality records for juvenile Oncorhynchus tshawytscha exposed to untreated Fraser River water for varying periods and subsequently incubated in Ceratomyxa shasta-free water.

Period of exposure (1983)	Number of days exposed	Number of fish exposed	Mortalities[a] by week[b] during incubation							Total fish dead
			1	2	3	4	5	6	8	
June 17 - 23	6	22	3+0+0= 3	8+0+0= 8	1+0+0= 1	1+1+3=5	0+0+3=3	0+0+1=1	0+1+0=1	13+2+7=22
June 17 - 23	6	24	14+0+0=14	8+0+0= 8	-	0+0+1=1	0+0+1=1	-	-	22+0+2=24
June 23 - 30	7	19	-	1+0+0= 1	3+9+0=12	0+2+2=4	0+2+0=2	-	-	4+13+2=19
June 17 - 30	13	24	-	3+3+2= 8	1+6+9=16	-	-	-	-	4+9+11=24
June 30 - July 7	7	26	1+0+0= 1	-	1+7+8=16	0+3+6=9	-	-	-	2+10+14=26
June 17 - July 7	20	25	-	0+9+9=18	0+2+5= 7	-	-	-	-	0+11+14=25
July 7 - 14	7	26	1+0+0= 1	7+10+0=17	0+8+0= 8	-	-	-	-	8+18+0=26
June 17 - July 14	27	25	2+19+4=25	-	-	-	-	-	-	2+19+4=25
July 14 - 21	7	23	1+0+0= 1	3+4+0= 7	1+10+3=14	0+0+1=1	-	-	-	5+14+4=23
July 21 - Aug. 4	14	26	0+15+2=17	0+6+3= 9	-	-	-	-	-	0+21+5=26
Aug. 4 - 15	11	23	1+8+1=10	1+12+0=13	-	-	-	-	-	2+20+1=23

[a] Recorded as: number of fish with no C. shasta detected in scrapings from the posterior intestine + number of fish with trophozoites only + number of fish with trophozoites, sporoblasts, and/or spores=total number of fish mortalities.

[b] No fish died during week 7.

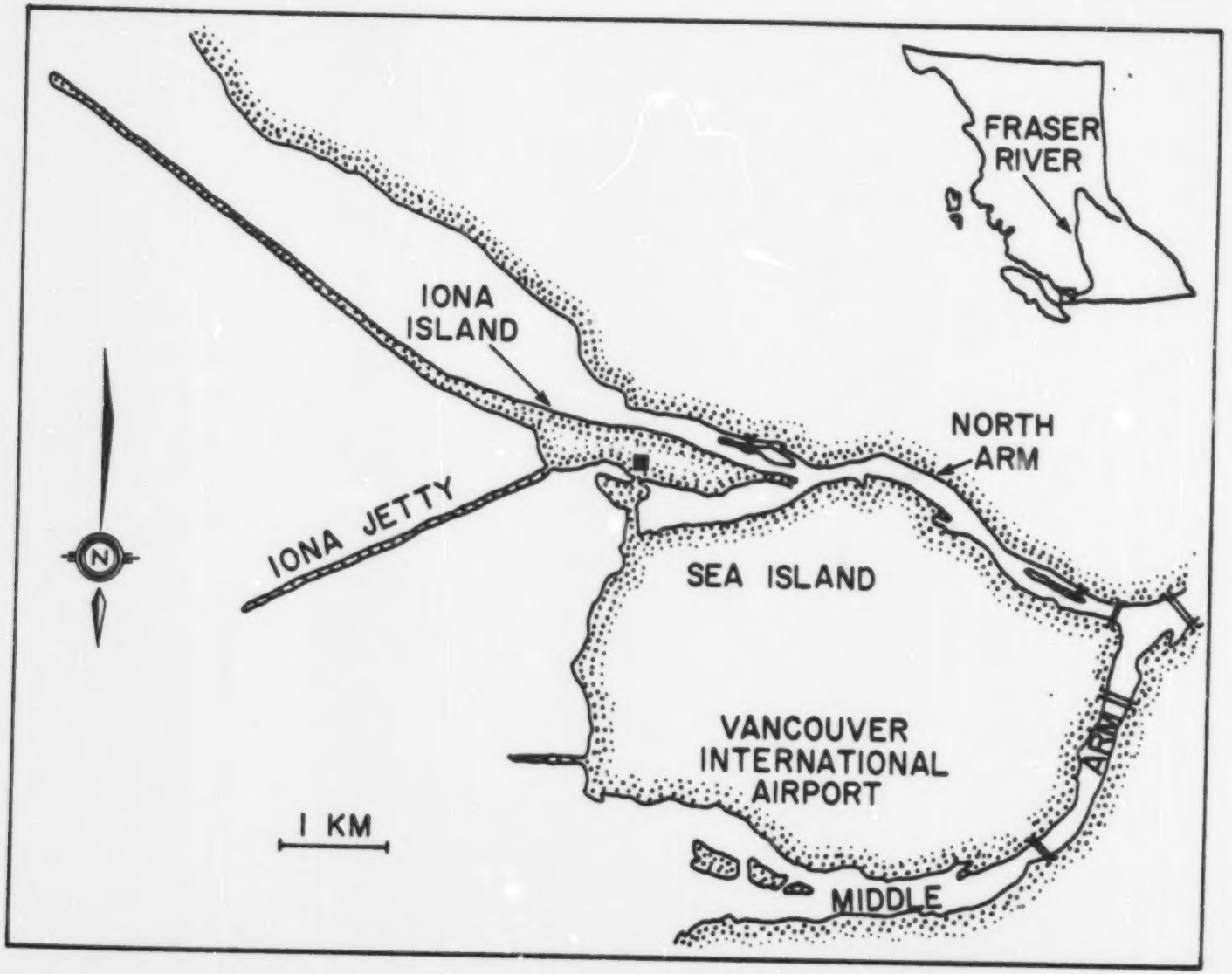

Figure 1. Study area. A. Iona Island on the North Arm of the Fraser River showing the location of the sewage treatment plant (solid square). B. Inset map of British Columbia, Canada indicating study area in the lower Fraser River by an open square.